Chapter 8

Relevance of the CDE and DDC Mouse Models to Study Ductular Reaction in Chronic Human Liver Diseases

Laure-Alix Clerbaux, Noémi Van Hul,
Annette S.H. Gouw, Rita Manco,
Regina Español-Suñer and Isabelle A. Leclercq

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.69533

Abstract

The liver has the remarkable capacity to regenerate through cellular division of hepatocytes. However, following severe injuries that abrogates the replicative capacity of hepatocytes some immature-like cells proliferate around the portal area and invade the parenchyma in a process known as ductular reaction (DR). In humans, DR is observed in virtually all chronic liver disorders although the morphological patterns may vary. DR biology has gained considerable interest because of potential contribution to hepatic cell restoration, fibrosis or carcinogenesis. In humans, observational studies are available but experimental manipulations and lineage tracing are impossible. Animal models represent thus valuable tools to explore such questions. Feeding rodents a choline-deficient, ethionine-supplemented diet (CDE) or a diet enriched in 3,5-diethoxycarboncyl-1,4-dihydrocollidine (DDC) are the most popular models to study DR. They are often used equivalently in the literature although the aspects and outcome of the DR are different and model-specific. Here, we describe experimental procedures and the pathophysiological mechanisms at play; we describe the hepatic lesions and highlight the unique character of DR phenotype, proliferation, lineage commitment and microenvironment in each model. We then compare the models with DR phenotype in human pathologies.

Keywords: liver progenitor cells, ductular reaction, CDE, DDC

1. Introduction

In a healthy liver, hepatocytes are quiescent long-lived cells. Upon mild to moderate hepatocellular injury or depletion, hepatocytes self-duplicate to restore the liver mass. However,

when there is a massive cell loss or a continuous damage to mature hepatic cells, overwhelming the replicative capacity of the remaining hepatocytes, expansion of immature-like cells is observed at the interface between the portal area and the parenchyma in a process called ductular reaction (DR). Expression of biliary markers is a hallmark of DR cells, but nevertheless, DR constitutes a heterogeneous population of proliferating cells ranging from immature stem-like cells to more committed cells with an intermediate hepatobiliary phenotype [1–4]. Cells of the DR are also called liver progenitor cells (LPC) as they have been shown to differentiate into both hepatocytes and cholangiocytes lineages in culture (reviewed in Ref. [5]). In normal livers, no DR are usually observed and LPC are seen, in two-dimensional tissue sections, as single cells located mainly in the canal of Hering, which represents the connection between the smallest ramifications of the biliary tree and the hepatocyte canalicular system [6, 7]. DR/LPC and biliary cells cannot strictly be distinguished at the histological level but based on their location and morphological differences [8]. In a three-dimensional viewpoint, DR and the biliary tree constitute together a contiguous heterogeneous epithelial structure [9]. In humans as in rodents, the histological and morphological patterns of DR vary according to injurious settings and their lineage commitment toward hepatocytes or cholangiocytes has been related to the primary site of cell loss or dysfunction [10].

Over the past decade, there has been a considerable interest in understanding DR/LPC biology. LPC are indeed seen as a potential reservoir for mature hepatocytes. Understanding the nature and differentiation process of LPC may generate cells for liver-cell therapy, which is increasingly under demand due to organ shortage for liver transplantation. Moreover, DR has also been postulated to trigger portal fibrosis [11]. Unraveling the potential mediators of DR could therefore be of great interest to modulate progression of profibrogenic reaction observed in many chronic liver diseases.

Several rodent models of liver injury associate with a DR and are instrumental to study the LPC response and its implication in liver regeneration and wound healing. These models, as in human liver diseases, exhibit a large variety of DR/LPC patterns with different morphological features, kinetics of response, and differentiation potential. The models of liver injury with DR generally combine the damage and loss of epithelial cells (hepatocytes and/or cholangiocytes) with the inhibition of the proliferative capacity (replicative senescence) of mature epithelial cells. Toxins [12, 13], carcinogens [14, 15], or modified diets [16, 17] have been used to induce cell injury, either alone or associated with surgical removal of part of the liver to amplify liver cell depletion. Ethionine, 2-acetylaminofluorene (AAF), and retrorsine are used to block the ability of mature epithelial cells to divide and prevent them from contributing to the liver regeneration process. In mice, dietary manipulations are regarded as convenient, efficient, and reproducible models to induce a robust DR, without need for animal handling, repeated injections, or surgical manipulation. The two most popular dietary DR models are a choline-deficient diet supplemented with ethionine in the drinking water (CDE) or a diet enriched in 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC).

In the literature, the DDC and CDE models are often used equivalently to study the LPC response and their role in tissue repair. However, DR in those two models exhibits major

http://dx.doi.org/10.5772/intechopen.69533

etiological and phenotypical differences. Such differences recapitulate the specificity of the pathophysiological responses to distinct injurious processes. DR activation, expansion, and capacity for differentiation are dictated by the nature of the cellular injury and by the differential microenvironment changes.

In this chapter, we will first describe the pathophysiological mechanisms at play in each model and the experimental procedures to induce DR with the CDE or DDC diet. A description of the hepatic lesions in terms of the cellular compartment injured after CDE and DDC feeding and highlight of the unique character of each model with regard to the DR phenotype, proliferation, lineage commitment, and microenvironment will be explored. Finally, the relevance of these models to study and understand the diversity of DR seen in human chronic liver diseases will be addressed.

2. The CDE model

2.1. The CDE model of hepatocellular injury: pathophysiological mechanisms and DR phenotype

The CDE model consists of *ad libitum* administration of a choline-deficient diet together with procurement of ethionine in the drinking water. Choline is provided by food intake and contributes to the structural integrity and signaling function of cell membranes. A choline withdrawal leads to a decreased synthesis of phosphatidylcholine, a phospholipid crucial for cell membrane and a major building stone of the very low-density lipoprotein particles produced by hepatocytes to export triglycerides. Choline deficiency causes intracytoplasmic fat accumulation, hepatocyte dysfunction, and cell damage [18, 19]. Such (extensive) hepatocellular damage results in high hepatocyte replication ratio, causing their exhaustion and restraining the production of hepatic drug metabolism-related enzymes [20]. Ethionine, a synthetic amino acid, specifically targets the hepatocytes in which, when provided in large excess, it competes with its naturally occurring analog methionine. Competition of ethionine with methionine favors the synthesis of S-adenosyl ethionine (SAE) instead of S-adenosyl methionine (SAM). Consequently, an ethyl group is transferred instead of a methyl group in methylation reactions hereby generating abnormal proteins, lipids, RNA, and DNA molecules, which results in hepatocytic cell damage [21]. Prolonged feeding with ethionine produces liver tumors with extensive LPC proliferation [22]. However, administration of ethionine in supplement to a choline-deficient diet greatly shortens the time required for LPC proliferation [22]. Although, the exact mechanism of action of CDE-induced injury is not well known, it appears that the combined administration of ethionine with choline-deficient chow induces a liver injury in which the hepatocytes are specifically targeted and the replication of the surviving hepatocytes is inhibited [23]. Hepatocyte proliferation to replace damaged liver cells is prevented and activation of the LPC compartment ensues. Several publications characterized the kinetics of the LPC response and liver damage to CDE [24–27]. Briefly, short-term CDE feeding results in steatosis, inflammation, LPC expansion (DR), and fibrosis that progress

in parallel. Cirrhosis and hepatocellular carcinoma may be observed in long-term studies. We intend to describe and analyze in depth the morphology and differentiation capacity of DR after 3 weeks of CDE (except when specified otherwise), at a time when pathological damages are installed and DR robustly established.

After 3 weeks, CDE livers are pale with signs of steatosis throughout the parenchyma. Liver weight is comparable or slightly lower than the deep-red control livers (**Figure 1A** and **B**). Signs of hepatocellular injury are observed with necrotic and apoptotic hepatocytes while bile ducts appear normal within the portal triad (**Figure 1G**) [23, 28]. Also, serum alanine aminotransaminases are increased while bilirubin levels are in the near normal range, indicative of hepatocytic damage (**Figure 1D** and **E**).

In the CDE model, DR expansion, seen on two-dimensional (2D) sections by staining with a biliary marker such as cytokeratin (CK) 19, is observed arising from the portal area and invading progressively the parenchyma (**Figure 1G**). First observable after approximately 1 week of CDE feeding, the DR progressively amplifies to a maximum around 3–4 weeks [23, 24, 29]. DR cells are small cells with a high nuclear-to-cytoplasm ratio, usually uniform in size with a fusiform shape and oval nuclei. On 2D liver sections, they are found as individual cells, grouped in multifocal clusters or organized in a single or double row of cells forming arborizing structures (**Figure 1**) [24]. Architectural three-dimensional (3D) analysis of the biliary tree remodeling in response to CDE reveal that DR are connected to the pre-existing bile ducts and that biliary branches intricately split around the portal vein with a random directionality [9]. Moreover, plastination of the bile duct system reveal a denser biliary network after CDE feeding (**Figure 1J**).

Finally, with regard to LPC capacity of differentiation *in vivo*, DR cell-tracking experiments using different transgenic mouse models [23, 28, 30] indicate that, upon CDE diet, a small number of DR cells do differentiate into hepatocytes: in the process LPC lose biliary markers, grow in size, and acquire mature hepatocyte morphological features and functional proteins. Although differentiation is consistently reported, only few DR-derived hepatocytes are reported in this model (<2.5% of hepatocytes).

2.2. The CDE model: practical aspects

Although being widely used, the CDE model is difficult to handle and researchers are confronted with difficulties and ethical issues due to variability in the LPC response, well-being of the animals, morbidity, and mortality. Here, we will review several factors influencing the LPC response to the model. These parameters must be taken into account and controlled to strengthen the model and provide reproducibility.

2.2.1. Dietary variables

Rodent food manufacturers can easily provide food in which the choline content is strictly controlled. Although low choline dietary content could be used [31], we will describe here a model using dietary choline deficiency. The second parameter to adjust for is ethionine supplementation. In the literature, the amount of ethionine in the water varies from 0.05 to 0.165% (wt/vol).

http://dx.doi.org/10.5772/intechopen.69533

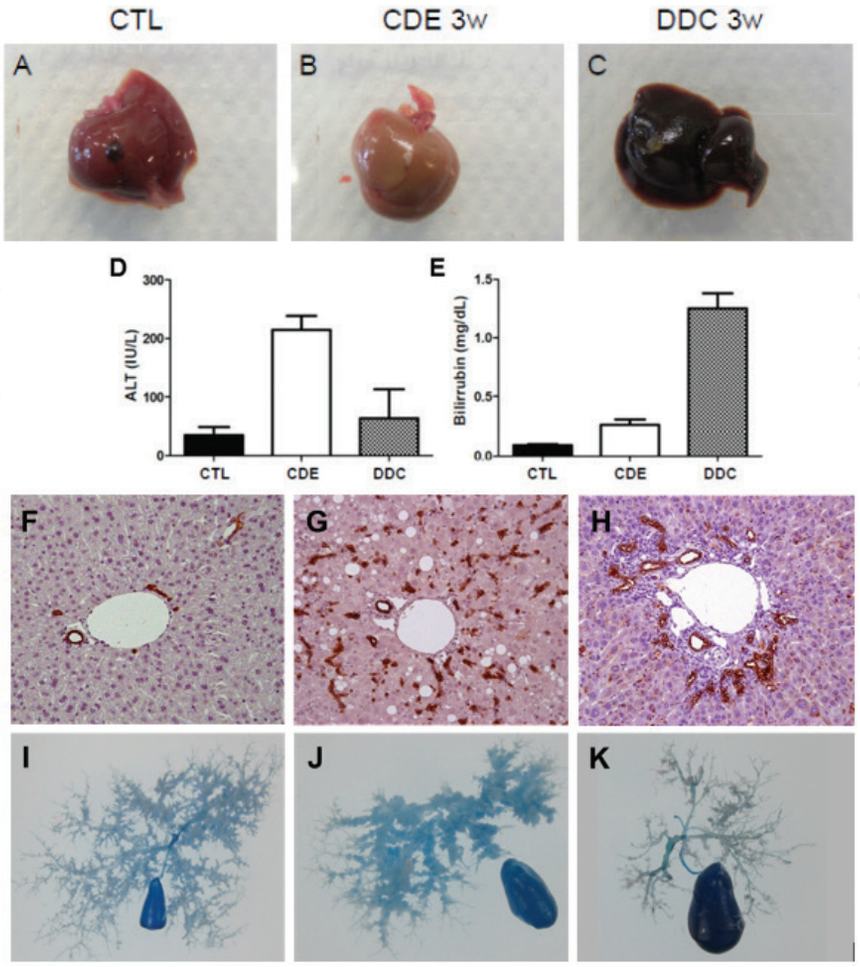

Figure 1. Pathophysiological mechanisms and DR phenotype in the CDE and DDC models. Livers retrieved from control mice (A), mice receiving the CDE (B), or DDC diet (C) for 3 weeks. Serum biochemical measurements for total aminotransferase (ALT) (D) and bilirubin (E) showed increased ALT and near normal bilirubin in the CDE model and increased serum bilirubin with slightly elevated ALT levels in DDC, indicative of hepatocellular damage upon CDE treatment and of primarily biliary injury after DDC diet. Liver sections stained with anticytokeratin 19 (CK19) in control (F), CDE (G), and DDC (H) livers after 3 weeks of diet. In control, CK19 staining reveals bile ducts and LPC as isolated cells close to the periportal tract. In CDE livers, besides bile ducts, DR CK19+ cells are strongly increased in number, forming cells organized in filaments expanding inside the lobule. After DDC feeding, in addition to the larger preexisting bile ducts, CK19+ newly formed DR structures are composed of small cuboidal cells, irregular in size and shape accumulated around the portal area. Plastination of the bile duct system reveals delicately structured biliary tree in control mice (I), a denser biliary network after CDE feeding (J), and dilatation of intrahepatic bile ducts in DDC-fed mice (K).

Mice are not fond of ethionine (due to bad smell), and usually decrease water consumption. This makes it difficult to control effective ethionine intake. Addition of 5% sucrose, choline-free orange juice, or fruit syrup is sometimes used to increase the attractiveness of the drink and this is most of the time not reported in the experimental protocol [9, 23, 28, 29, 32–34]. Ethionine smell increases with exposure to the ambient air and we found that we could maintain stable water intake by replacing ethionine-containing water by a fresh solution every day. Although this sounds trivial, control over ethionine solution consumption is crucial as both variation in ethionine intake and (severe) dehydration may influence LPC response and induce large interindividual variation in the model. To circumvent this, Passman et al. also propose to include ethionine in the chow [29].

2.2.2. Mouse variables

LPC response and morbidity vary according to weight and age of the mice at the time of introduction of the CDE diet. Mice above 25–30 g will be quite resistant to the diet and if they are too old, perhaps because of loss of cell plasticity, LPC response will be discrete. In parallel, if they are too young or too little (<15 g) at the time of dietary exposure, toxicity and ensuing mortality might be excessively high. With the administration of the CDE diet to mice of 6 weeks of age and with a body weight between 18 and 20 g, we and others show a substantial and reasonably reproducible LPC response while maintaining the well-being of the animals [24, 28, 29, 35]. Mice are experiencing the most severe effects of the diet during the first week of administration. Following the first few days, significant weight loss is observed, often associated with mortality [29, 36]. Approximately 1 week after the onset of the CDE treatment, the mice adapt, regain weight, and show (normal) physical activity and behavior. Thus, by respecting the simple rules proposed above, body weight loss may be limited to 10% of the starting body weigh during the first week with weight stabilization thereafter. Importantly, sensitivity to the dietary regimen and magnitude of liver damage and LPC reaction largely vary according to the genetic background of the mice [17]. This imposes the use of an appropriate control group (best being littermates) when comparing the effect of gene deletion or addition in genetically modified animals.

3. The DDC model

3.1. The DDC model of biliary injury: pathophysiological mechanisms and DR phenotype

The DDC model consists of *ad libitum* administration of a diet enriched with the porphyrinogenic agent 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) with normal water. Exposure to a DDC diet provokes the inhibition of the mitochondrial enzyme ferrochelatase, catalyst of the insertion of ferrous iron into protoporphyrin IX to form heme, leading to progressive accumulation of protoporphyrin. This brown pigment first accumulates in the cytoplasm of parenchymal cells and in Kupffer cells. Because of its hydrophobic nature, the excess of protoporphyrin can only exit the liver through biliary excretion, leading to precipitation of this poorly soluble molecule in bile canaliculi and bile ducts, forming crystals increasing in size and number [37]. After 3 weeks of DDC diet, accumulated pigments plug and obstruct the lumen of the smaller branches of the biliary tree and confer a dark coloration to the liver (**Figure 1C**). Bile ducts, usually recognizable as monolayer rings of small cuboidal cholangiocytes delineating a central lumen, show profound morphological alterations while hepatocytes have a normal appearance except for pigment coloration (**Figure 1H**). This indicates that the DDC dietary regimen mostly damage the biliary system, which is additionally supported by increased serum bilirubin (**Figure 1E**). We observed moderately elevated transaminases levels (two to threefold time, **Figure 1D**) although another group reports higher transaminase levels after DDC feeding [16]. In later stages, livers

in DDC-fed mice develop pericholangitis and periductal onion skin-like fibrosis. Our discussion here analyzes DR morphology and microenvironment after 3 weeks of DDC feeding.

In the DDC livers, bile duct damage is associated with a biliary response in which dysmorphic cholangiocytes proliferate in the portal area. In all portal tracts, DR expands as multiple small pseudo-ducts arising next to the larger preexisting bile ducts (**Figure 1H**). These newly formed ductular structures are composed of small cuboidal or more cylindrical cells, irregular in size and shape, assembled in tube-like structures outlining a lumen in most cases, sometime plugged by porphyrin crystals. In contrast to infiltrating DR in CDE livers, DR expansion observed in DDC livers remains enclosed within the portal mesenchyme. No parenchymal invasion crossing the boundaries of portal mesenchyme was observed nor did those reactive cells, always observed as a cluster, adopt a phenotype supporting migration. However, the portal mesenchyme extends and may bridge distant portal spaces (**Figure 1H**). 3D biliary analysis of DDC livers identifies branches randomly directed around the portal vein, connected to the biliary tree but forming apparent distinct structures from the large-diameter bile ducts [9]. Moreover, 3D plastination of the DDC-fed mouse confirms slight focal dilatation of intrahepatic bile ducts and porphyrin plugs while biliary network seems to be less dense [16] (**Figure 1K**).

Finally, concerning LPC capacity of differentiation, upon DDC-induced injury, there is no evidence that cells of the neo ducts undergo hepatocytic cell differentiation [23, 28, 30]. When animals are reversed to a standard chow after DDC diet, the degree of DR expansion decreases, but still with no evidence that DR cells differentiate into hepatocytes. Because a specific LPC marker, that is, exclusively expressed in LPC and not in cholangiocytes, is lacking, we are currently unable to experimentally address the contribution of LPC to biliary regeneration *in vivo* during disease evolution. We can, however, hypothesize that, if not entirely supported by proliferation of mature cholangiocytes, LPC located at the most proximal part of the biliary tree contribute to neo duct formation in the DDC model [9].

3.2. The DDC model: practical aspects

Contrasting with the CDE model, the DDC model is robust and reproducible and has little impact on animal welfare. In all studies, diet (standard rodent chow) is supplemented with 0.1% (wt/wt) of DDC. Similarly, the different mouse strains tested so far develop comparable hepatic phenotype to DDC feeding [16] although differences in susceptibility and kinetics of the response might be expected according to strains. Of note, DDC diet applied to rats does not induce any LPC response [38].

4. Microenvironment-regulating DR expansion and differentiation

The literature brings every day new evidence that the orchestrated interplay between proliferating hepatocytes or cholangiocytes, extracellular matrix-producing myofibroblasts, inflammatory cells (such as macrophages, neutrophils, or lymphocytes), and endothelial cells is pivotal in the regulation of DR expansion and differentiation. We will thus compare the microenvironment accompanying DR in the CDE versus DDC model.

4.1. Extracellular matrix, collagen, and laminin

Extracellular matrix and collagen deposition associates with DR. In the CDE model, a thin and loose web of collagen fibers is associated with invading DR cells, while collagens in DDC livers thicken the portal mesenchyme and abundant extracellular matrix accumulates in clots or thick concentric layers around the neo-formed pseudo-ductular structures (**Figure 2A–D**).

The localization of myofibroblasts, the cells chiefly involved in matrix synthesis and remodeling, adopted a pattern similar to that of the collagen deposition in both models, meaning that DR is at all times associated with myofibroblasts. In CDE livers, myofibroblasts chaperone the DR cells while they penetrate deep into the liver lobule (**Figure 2E**). Conversely, in the DDC model, myofibroblasts densely populate the portal mesenchyme and accumulate rather concentrically around DR (**Figure 2F**).

Laminin is a component of the basal membrane delineating the basal pole of cholangiocytes. Basement membrane is essential to establish the cholangiocytes polarity and to support a tubular structure with a lumen [39]. By contrast, hepatocytes do not lie on a basement membrane. In the CDE model, DR is anchored onto a laminin-rich basal membrane intermingled with collagen. This layer of laminin has been proposed to maintain the immature/biliary phenotype of DR cells and to provide a holding structure facilitating migration of DR into the lobular parenchyma in the CDE model [28, 40]. Moreover, decreased density of laminin and extracellular matrix in CDE livers is associated with enhanced hepatocytic differentiation of DR cells [28]. Indeed, during DR differentiation process, DR cells progressively lose contact with the laminin-rich basement. And when animals are reversed to a standard chow (supply of choline and cessation of ethionine administration) after CDE exposure, the injury reverses, DR, extracellular matrix, and laminin deposition progressively lessen, and concomitantly the number of DR-derived hepatocytes increases.

In DDC livers, laminin deposits as thin basal membrane outlining the DR in a pattern similar to that seen around normal bile ducts, with collagen stacked as separate sheets encircling newly formed DR.

4.2. Inflammatory environment

In response to liver injury, Kupffer cells, the hepatic macrophages, activate and participate to the recruitment of the inflammatory reaction. In CDE livers, enlarged and proliferative Kupffer cells are strongly associated with invading DR within the parenchyma while no portal inflammation is observed [41]. DDC-induced proliferation of the ducts is accompanied by a dense macrophage and neutrophil granulocytic infiltrate around small and larger bile ducts, further supporting that biliary structures are first concerned by the injurious and healing responses in this model [16].

Kupffer cells do not influence DR expansion but modulate its invasive behavior and its specification, through modulation of the density of extracellular matrix as well as via Notch and Wnt signaling pathways [41, 42]. Numb, a direct transcriptional target of Wnt and a negative regulator of Notch, is downregulated in LPC during biliary regeneration, promoting

http://dx.doi.org/10.5772/intechopen.69533

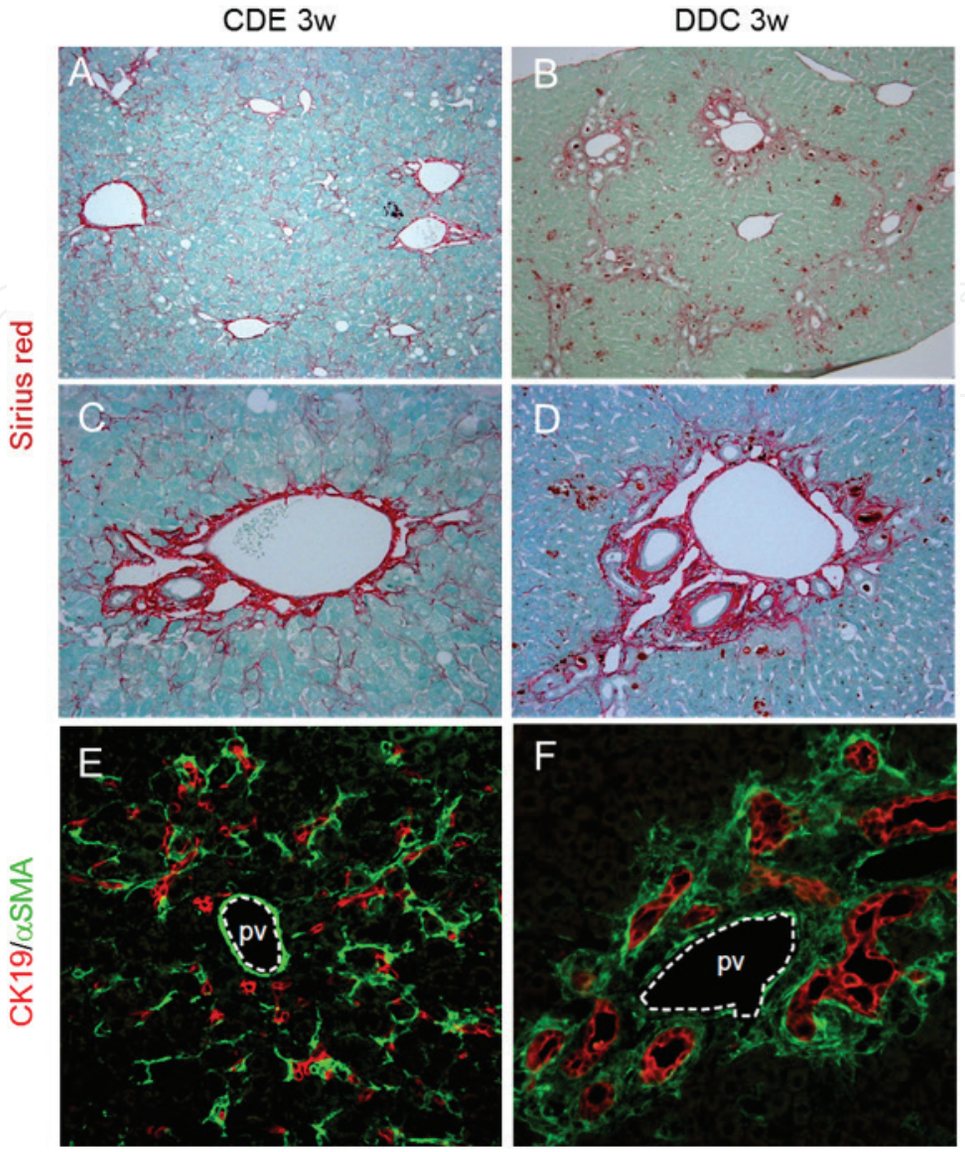

Figure 2. Comparison of the extracellular matrix deposition and the myofibroblast expansion between the CDE and DDC models. Liver sections obtained from mice receiving the CDE (A, C, E) or DDC diet (B, D, F) were stained with Sirius red to highlight fibrillary collagen (A-D), or αSMA and CK19 expression (E and F). In CDE livers, a collagen meshwork covers the whole parenchyma (A) with fibers elongating from the portal area into the lobule (C). In DDC livers, collagen fibers accumulate around the portal area to shape the portal mesenchyma (D), delimiting the boundaries of DR (F). At lower magnification, portal-portal bridging is observed (B). αSMA+ myofibroblasts show a distribution pattern similar as the collagen deposition. αSMA+ myofibroblasts infiltrate the lobule, chaperoning CK19+ DR cells in the CDE model (E), while in the DDC model, αSMA+ myofibroblasts rather accumulate concentrically around the DR structures (F).

biliary specification via the Notch pathway. While during hepatocyte regeneration, macrophage-derived canonical Wnt signaling maintains Numb within LPC and Notch signaling is reduced, promoting hepatocyte specification [42].

Experiments performed in lymphocyte-deficient mice fed on CDE suggest that natural killer cells and T-cells participate also to LPC expansion, presumably through their proinflammatory cytokine production [43]. Moreover, TNF-like weak inducer of apoptosis (TWEAK), produced

by T-cells and activating its receptor fibroblast growth factor-inducible 14 (Fn14), is suggested to be an exclusive LPC mitogen. After both CDE and DDC treatment on Fn14 knockout mice, a significant reduction of the LPC response is observed [31, 44].

4.3. Nearby endothelial cells

As described above, DR requires a typical niche provided by extracellular matrix-producing and inflammatory cells, which are located in the sinusoids closely adjacent to DR. Additionally, sinusoidal endothelial cells themselves could also have an important role in regulating DR. Signaling molecules specifically expressed within the endothelial compartment of the central vein have been shown to have a crucial role in liver zonation [45]. Moreover, in another model of liver injury, hepatocytes divide along the closest microvessel as order principle to restore liver architecture [46]. Either a signaling or a guiding role of endothelial cells on LPC response could be envisaged. However, so far, no experiments have been done to study endothelial regulation of DR in the CDE or DDC model.

5. Comparison of the CDE and DDC models with chronic human liver diseases, HCV and PSC, respectively

In humans, DR is seen in most chronic liver injury, irrespective of the etiology. Historically, DR has been categorized on morphology into "*typical*" and "*atypical*" DR, based on rodent studies [47]. *Typical* DR have a lumen lined by cuboidal cells and are the result of proliferation of preexisting ductules, in analogy with the DR seen after biliary obstruction, while *atypical* describes thin, elongated structures that extend into the lobules and lack discernible lumen as preferentially seen after hepatocytic damage. Therefore, DDC diet best models *typical* DR, while CDE diet replicates pathological pattern of *atypical* DR. However, this dichotomic classification was discouraged some years ago because it could not readily accommodate the range of patterns seen clinically [10]. Another classification schemes attempted to integrate the histologic features, inciting disease and immunophenotyping of DR [48, 49]. However, not all DR fit in this classification, and especially not when biliary obstruction becomes chronic (as in the DDC livers). So far, there is a lack of consensus regarding DR classification in humans, as DRs are diverse, covering a spectrum of features rather than clear subphenotypes [10].

However, based on histological analysis, the DR phenotype in the CDE model resembles the one observed in human chronic Hepatitis C virus (HCV) infection depicting portal fibrosis and in a series of autoimmune hepatitis (AIH) (**Figure 3A–D**). HCV- and AIH-associated DRs have only a vague or no lumen, and comprise small elongated cells with little cytoplasm extending in the periportal parenchyma and associated with dense collagen fibers [10, 11, 50]. In HCV, the extension of DR into the parenchyma and DR severity correlate with the severity of fibrosis and the inflammatory activity, supporting that extracellular matrix and inflammatory signals influence DR [50]. DR in AIH has been proposed to represent a regenerative response as DR persists after the inflammatory activity subsided following immunosuppressive treatment [51]. At the early stage of fibrosing, cholestatic variants of coinfection with hepatitis B and C, expanded DR into the hepatic parenchyma also resemble the DR phenotype seen in CDE livers [10, 52]. With regard

http://dx.doi.org/10.5772/intechopen.69533

to LPC fate, the observation of a phenotypic continuum between DR cells and hepatocytes in the livers of patients suffering from HCV supports differentiation of LPC toward hepatocytes [4, 50]. Besides hepatitis, the CDE diet also recapitulates features of the DR associated with lipid accumulation (steatosis) as in chronic alcoholic and nonalcoholic fatty liver diseases [11, 29, 42, 53].

The DR pattern seen in DDC livers is more comparable to that of chronic fibrosing cholangitis such as primary sclerosing cholangitis (PSC) and primary biliary cholangitis (PBC) with DR proliferation restricted within the portal area and accompanied by concentric periportal fibrosis (**Figure 3E** and **F**). In these diseases as in DDC, the primary damage is directed toward cholangiocytes. Intrahepatic bile duct destruction and ductopenia seen in advanced PBC and the fibrous obliterative lesions of PSC do not occur in the DDC model, a phenomenon most likely related to the specific immune component of PBC and PSC which is lacking in the DDC model.

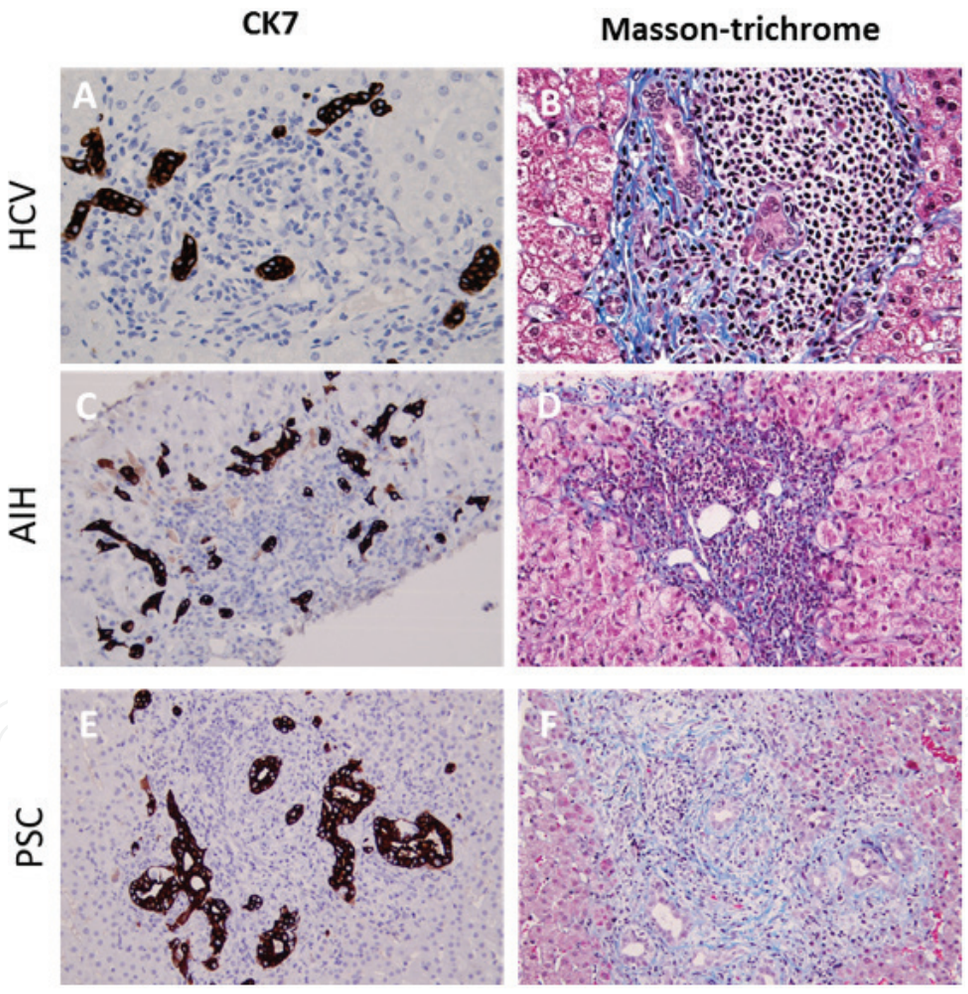

Figure 3. DR observed in human chronic hepatitis C infection and in primary sclerosing cholangitis. Liver stained with anticytokeratin 7 (CK7) and Masson-trichrome of an HCV case with mild inflammation (A and B), AIH with moderate inflammation (C and D), and PSC with cholangitis, edema, and portal fibrosis (E and F). HCV- and AIH-associated CK7+ DRs (A and C) have only a vague or no lumen and comprise and elongated cells with little cytoplasm extending in the periportal parenchyma and associated with dense collagen fibbers (B and D). While the CK7+ ductular proliferation seen in PSC (E) is enclosed in portal mesenchyma and concentric periductular fibrosis occurs (F).

As mentioned above, the Notch and Wnt signaling pathways are involved in the divergence of DR cells fate toward hepatocyte or biliary cells observed in response to CDE versus DDC. Similarly, in human diseases, prevalence of Notch signaling, driving biliary phenotype, is strong in PSC while the expression of Numb, a negative regulator of Notch, is more elevated in HCV samples compared to PSC [54]. Moreover, β-catenin, a component of the Wnt pathway, is found within the cytoplasm and nucleus of human DR cells of HCV-infected livers, signing enhanced Wnt signaling and promoting hepatocyte regeneration, whereas in PSC, β-catenin is predominantly localized to the cell surface, suggesting low activation of the canonical Wnt signaling pathway promoting biliary regeneration [42].

6. Conclusion

In summary, the CDE diet targets specifically hepatocytes and induce DR-containing elongated cells of an undifferentiated and migration-supporting phenotype expanding from portal tracts into the parenchyma. Myofibroblast activation and extracellular matrix deposition precedes this cell expansion, and a laminin-rich sheet sustains those DR while macrophages associate with invading DR. In the DDC model, accumulating protoporphyrin obstructs the hepatobiliary system leading to biliary damage and resulting in highly proliferative cells forming bile duct-like structures remaining restricted to portal mesenchyme, delineated by a thin layer of laminin and accompanied by dense portal inflammation. Moreover, cell-tracking experiments revealed that DR cells are able to generate a small number of functional hepatocytes after CDE but not after DDC exposure. Finally, the DR phenotype and signaling pathways involved in LPC differentiation in the CDE model mirrors the one observed in chronic HCV infection presenting signs of fibrosis and autoimmune hepatitis, while the DDC model could be used to study biliary injury such as PSC or PBC in humans. We believe that characterization of the most widely used dietary DR mouse models will help our understanding of the diversity of DR patterns observed in humans and will help the researchers to select the appropriate model in relation to the specific question addressed.

Author details

Laure-Alix Clerbaux[1], Noémi Van Hul[2], Annette S.H. Gouw[3], Rita Manco[1], Regina Español-Suñer[4] and Isabelle A. Leclercq[1]*

*Address all correspondence to: isabelle.leclercq@uclouvain.be

1 Laboratory of Hepatogastroenterology, Université Catholique de Louvain, Brussels, Belgium

2 Cell Division and Cancer Laboratory, A*STAR, Singapore

3 Department of Pathology and Medical Biology, University of Groningen, Groningen, The Netherlands

4 Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California, San Francisco, California, USA

References

[1] Lowes KN, Brennan BA, Yeoh GC, Olynyk JK. Oval cell numbers in human chronic liver diseases are directly related to disease severity. American Journal of Pathology. 1999;**154**(2):537-541

[2] Roskams TA, Libbrecht L, Desmet VJ. Progenitor cells in diseased human liver. Seminars in Liver Disease. 2003;**23**:385-396

[3] Theise N, Saxena R, Portmann BC, Thung SN, Yee H, Chiriboga L, et al. The canals of Hering and hepatic stem cells in humans. Hepatology. 1999;**30**:1425-1433

[4] Zhang L, Theise N, Chua M, Reid LM. The stem cell niche of human livers: Symmetry between development and regeneration. Hepatology. 2008;**48**:1598-1607

[5] Riehle KJ, Dan YY, Campbell JS, Fausto N. New concepts in liver regeneration. Journal of Gastroenterology and Hepatology. 2011;**26**:203-212

[6] Duncan AW, Dorrell C, Grompe M. Stem cells and liver regeneration. Gastroenterology. 2009;**137**(2):466-481

[7] Kuwahara R, Kofman AV, Landis CS, Swenson ES, Barendswaard E, Theise N. The hepatic stem cell niche: Identification by label-retaining cell assay. Hepatology. 2008;**47**(6): 1994-2002

[8] Saxena R, Theise N. Canals of hering: Recent insights and current knowledge. Seminars in Liver Disease. 2004;**24**(1):43-48

[9] Kaneko K, Kamimoto K, Miyajima A, Itoh T. Adaptive remodeling of the biliary architecture underlies liver homeostasis. Hepatology. 2015;**61**:2056-2066

[10] Gouw ASH, Clouston AD, Theise N. Ductular reactions in human liver: Diversity at the interface. Hepatology. 2011;**54**(5):1853-1863

[11] Clouston AD, Powell EE, Walsh MJ, Richardson MM, Demetris AJ, Jonsson JR. Fibrosis correlates with a ductular reaction in hepatitis C: Roles of impaired replication, progenitor cells and steatosis. Hepatology. 2005;**41**(4):809-818

[12] Dusabineza A, Van Hul KN, Abarca-quinones J, Starkel P, Najimi M, Leclercq IA. Participation of liver progenitor cells in liver regeneration : Lack of evidence in the AAF/PH rat model. Laboratory Investigation. 2012;**92**:72-81

[13] Kofman AV, Morgan G, Kirschenbaum A, Osbeck J, Hussain M, Swenson S, et al. Dose- and Time-Dependent oval cell reaction in acetaminophen-induced murine liver injury. Hepatology. 2005;**41**:1252-1261

[14] Factor VM, Radaeva SA, Thorgeirssont SS. Origin and fate of oval cells in dipin-induced hepatocarcinogenesis in the mouse. American Journal of Pathology. 1994;**145**(2):409-422

[15] Sell S. Heterogeneity and plasticity of hepatocyte lineage cells. Hepatology. 2001;**33**: 738-750

[16] Fickert P, Stöger U, Fuchsbichler A, Moustafa T, Marschall H-U, Weiglein AH, et al. A new xenobiotic-induced mouse model of sclerosing cholangitis and biliary fibrosis. American Journal of Pathology. 2007;**171**(2):525-536

[17] Knight B, Akhurst B, Matthews VB, Ruddell RG, Ramm GA, Abraham LJ, et al. Attenuated liver progenitor (oval) cell and fibrogenic responses to the choline deficient, ethionine supplemented diet in the BALB/c inbred strain of mice. Journal of Hepatology. 2007;**46**(1):134-141

[18] Lombardi B, Pani P, Schlunk FF. Choline-deficiency fatty liver: Impaired release of hepatic triglycerides. Journal of Lipid Research. 1968;**9**:437-446

[19] Zeisel SH, Da Costa K-A, Franklin PD, Alexander EA, Lamont JT, Sheard NF, et al. Choline, an essential nutrient for humans. The FASEB Journal. 1991;**5**(7):2093-2098

[20] Shinozuka H, Lombardi B, Sell S, Iammarino R. Early histological and functional alterations of ethionine liver carcinogenesis in rats fed a choline-deficient diet. Cancer Research. 1978;**38**:1092-1098

[21] Alix J-H. Molecular aspects of the in vivo and in vitro effects of ethionine, an analog of methionine. Microbiology Reviews. 1982;**46**(3):281-295

[22] Sell S. Comparison of liver progenitor cells in human atypical ductular reactions with those seen in experimental models of liver injury. Hepatology. 1998;**27**(2):317-331

[23] Shin S, Upadhyay N, Greenbaum LE, Kaestner KH. Ablation of Foxl1 Cre-labeled hepatic progenitor cells and their descendants impairs recovery from liver injury. Gastroenterology. 2015;**33**(1):395-401

[24] Van Hul N, Abarca-Quinones J, Sempoux C, Horsmans Y, Leclercq IA. Relation between liver progenitor cell expansion and extracellular matrix deposition in a CDE-induced murine model of chronic liver injury. Hepatology. 2009;**49**(5):1625-1635

[25] Akhurst B, Croager EJ, Farley-Roche CA, Ong JK, Dumble ML, Knight B, et al. A modified choline-deficient, ethionine-supplemented diet protocol effectively induces oval cells in mouse liver. Hepatology. 2001;**34**(3):519-522

[26] Davies RA, Knight B, Tian YW, Yeoh GCT, Olynyk JK. Hepatic oval cell response to the choline-deficient, ethionine supplemented model of murine liver injury is attenuated by the administration of a cyclo-oxygenase 2 inhibitor. Carcinogenesis. 2006;**27**(8):1607-1616

[27] Ochoa-Callejero L, Pérez-Martínez L, Rubio-Mediavilla S, Oteo JA, Martínez A, Blanco JR. Maraviroc, a CCR5 antagonist, prevents development of hepatocellular carcinoma in a mouse model. PLoS One. 2013;**8**(1):e53992

[28] Español-Suñer R, Carpentier R, Van Hul N, Legry V, Achouri Y, Cordi S, et al. Liver progenitor cells yield functional hepatocytes in response to chronic liver injury in mice. Gastroenterology. 2012;**143**(6):1564-1575.e7

[29] Passman AM, Strauss RP, Mcspadden SB, Finch-edmondson ML, Woo KH, Diepeveen LA, et al. A modified choline-deficient, ethionine-supplemented diet reduces morbidity and retains a liver progenitor cell response in mice. Disease Models & Mechanisms. 2015;**8**:1635-1641

[30] Rodrigo-Torres D, Affò S, Coll M, Morales-Ibanez O, Millán C, Blaya D, et al. The biliary epithelium gives rise to liver progenitor cells. Hepatology. 2014;**60**(4):1367-1377

[31] Tirnitz-parker JEE, Viebahn CS, Jakubowski A, Klopcic BRS, Olynyk JK, Yeoh GCT, et al. Tumor necrosis factor–like weak inducer of apoptosis is a mitogen for liver progenitor cells. Hepatology. 2010;**52**:291-302

[32] Tarlow BD, Finegold MJ, Grompe M. Clonal tracing of Sox9+ liver progenitors in mouse oval cell injury. Hepatology. 2014;**60**(1):278-289

[33] Schaub JR, Malato Y, Gormond C, Willenbring H. Evidence against a stem cell origin of new hepatocytes in a common mouse model of chronic liver injury. Cell Reports. 2014;**8**(4):933-939

[34] Yanger K, Knigin D, Zong Y, Maggs L, Gu G, Akiyama H, et al. Adult hepatocytes are generated by self-duplication rather than stem cell differentiation. Cell Stem Cell. 2014;**15**(3):340-349

[35] Lombardi B, Rao KN. Acute hemorrhagic pancreatic necrosis in mice. American Journal of Pathology. 1975;**81**:87-100

[36] Köhn-Gaone J, Dwyer BJ, Grzelak CA, Miller G, Shackel NA, Ramm GA, et al. Divergent inflammatory, fibrogenic, and liver progenitor cell dynamics in two common mouse models of chronic liver injury. American Journal of Pathology. 2016;**186**(7):1762-1774

[37] Meerman LEO, Koopen NR, Bloks V, Goor HVAN, Havinga R, Wolthers BG, et al. Biliary fibrosis associated with altered bile composition in a mouse model of erythropoietic protoporphyria. Gastroenterology. 1999;**117**:696-705

[38] Jelnes P, Santoni-rugiu E, Rasmussen M, Friis SL, Nielsen JH, Tygstrup N, et al. Remarkable heterogeneity displayed by oval cells in rat and mouse models of stem cell–mediated liver regeneration. Hepatology. 2007;**45**:1462-1470

[39] Tanimizu N, Kikkawa Y, Mitaka T, Miyajima A. α1- and α5-containing laminins regulate the development of bile ducts via 1 integrin signals*. The Journal of Biological Chemistry. 2012;**287**(34):28586-28597

[40] Lorenzini S, Bird TG, Boulter L, Bellamy C, Aucott R, Clayton E, et al. Characterisation of a stereotypical cellular and extracellular adult liver progenitor cell niche in rodents and diseased human liver. Gut. 2010;**59**(5):645-654

[41] Van Hul N, Lanthier N, Español-Suñer R, Abarca Quinones J, Van Rooijen N, Leclercq I. Kupffer cells influence parenchymal invasion and phenotypic orientation, but not the proliferation, of liver progenitor cells in a murine model of liver injury. American Journal of Pathology. 2011;**179**(4):1839-1850

[42] Boulter L, Govaere O, Bird T. Macrophage derived Wnt signalling opposes Notch signalling in a Numb mediated manner to specify HPC fate in chronic liver disease in human and mouse. Nature Medicine. 2012;**18**(4):572-579

[43] Strick-marchand H, Masse GX, Mary C, Santo JP Di. Lymphocytes support oval cell-dependent liver regeneration. The Journal of Immunology. 2008;**181**:2764-2771

[44] Jakubowski A, Ambrose C, Parr M, Lincecum JM, Wang MZ, Zheng TS, et al. TWEAK induces liver progenitor cell proliferation. Journal of Clinical Investigation. 2005;**115**(9):2330-2340

[45] Rocha AS, Vidal V, Mertz M, Kendall TJ, Charlet A, Okamoto H, et al. The angiocrine factor Rspondin3 is a key determinant of liver zonation report the aangiocrine factor Rspondin3 is a key determinant of liver zonation. Cell Reports. 2015;**13**(9):1757-1764

[46] Hoehme S, Brulport M, Bauer A, Bedawy E, Schormann W, Hermes M. Prediction and validation of cell alignment along microvessels as order principle to restore tissue architecture in liver regeneration. Proceedings of the National Academy of Sciences. 2010;**107**:10371-10376

[47] Alvaro D, Mancino MG, Glaser S, Gaudio E, Marzioni M, Francis H, et al. Proliferating cholangiocytes: A neuroendocrine compartment in the diseased liver. Gastroenterology. 2007;**132**:415-431

[48] Desmet VJ. Ductal plates in hepatic ductular reactions. Hypothesis and implications. I. Types of ductular reaction reconsidered. Virchows Archiv. 2011;**458**(3):251-259

[49] Turànyi E, Dezso K, Csomor J, Zsuzsa S, Paku S, Nagy P. Immunohistochemical classification of ductular reactions in human liver. Histopathology. 2010;**57**:607-614

[50] Libbrecht L, Desmet V, Van Damme B, Roskams T. Deep intralobular extension of human hepatic ` progenitor cells ' correlates with parenchymal inflammation in chronic viral hepatitis: can `progenitor cells ' migrate ? The Journal of Pathology. 2000;**192**:373-378

[51] Verdonk RC, Lozano MF, Berg AP Van Den, Gouw ASH. Bile ductal injury and ductular reaction are frequent phenomena with different significance in autoimmune hepatitis. Liver International. 2016;**36**:1362-1369

[52] Xiao S, Lu L, Wang HL. Fibrosing cholestatic hepatitis : Clinicopathologic spectrum, diagnosis and pathogenesis. International Journal of Experimental Pathology. 2008;**1**:396-402

[53] Tonkin JN, Knight B, Curtis D, Abraham LJ, Yeoh GCT. Bone marrow cells play only a very minor role in chronic liver regeneration induced by a choline-deficient, ethionine-supplemented diet. Stem Cell Research. 2008;**1**(3):195-204

[54] Spee B, Carpino G, Schotanus BA, Katoonizadeh A, Borght S Vander, Gaudio E, et al. Characterisation of the liver progenitor cell niche in liver diseases: Potential involvement of Wnt and Notch signalling. Gut. 2010;**59**:247-257